The effect of fission–fusion zoo housing on hormonal and behavioral indicators of stress in Bornean Orangutans (Pongo pygmaeus)

Amrein, Martin ; Heistermann, Michael ; Weingrill, Tony

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The Effect of Fission–Fusion Zoo Housing on Hormonal and Behavioral Indicators of Stress in Bornean Orangutans (*Pongo pygmaeus*)

Martin Amrein · Michael Heistermann · Tony Weingrill

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Abstract The welfare of captive animals could be improved if zoos were to place more emphasis on their species-specific needs. In the wild, orangutans live in a fission–fusion social system and have a semisolitary lifestyle. However, most zoos keep orangutans in permanent groups, which may be stressful for them. Apenheul Primate Park in Apeldoorn, Netherlands, houses 14 Bornean orangutans in a simulated fission–fusion social system. To assess how this housing system affects indicators of stress, we measured fecal glucocorticoid metabolites (fGCM) and determined proportions of self-directed behavior (scratching and autogrooming). We compared fGCM concentrations of the Apenheul animals to those of zoo orangutans kept in permanent groups. In addition, we tested the effect of group size, visitor number, sex, age, and change of group composition on fGCM concentrations and proportions of self-directed behavior in the Apenheul orangutans. In contrast to Bornean orangutans housed in permanent groups, we did not find a group size effect on fGCM or on self-directed behavior in Apenheul's fission–fusion housing system. In addition, fGCM concentrations in Apenheul orangutans increased significantly with visitor numbers. Visitor number also affected proportions of self-directed behavior, and mean proportions of scratching were positively correlated with mean values of fGCM concentrations. Although these results suggest that the fission–fusion housing system in Apenheul reduces the group size effect leading to social stress in Bornean orangutans, they also show that visitors are an important factor that needs to be mitigated if the well-being of captive primates and other zoo animals is to be improved.

M. Amrein (✉) · T. Weingrill
Anthropological Institute and Museum, University of Zurich, 8006 Zurich, Switzerland
e-mail: mail@martinamrein.ch

M. Heistermann
Endocrinology Laboratory, German Primate Center, 37077 Göttingen, Germany

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Introduction

Interest in the welfare of zoo animals has grown considerably in recent years (Hill and Broom 2009; Hosey *et al.* 2009; Maple 2007). Concerned with the condition of animals, zoos are increasingly keen to reduce sources of stress in captivity. In vertebrates, glucocorticoids secreted by the adrenal cortex are part of the natural stress response (Sapolsky 2002), and high glucocorticoid concentrations secreted over long periods of time potentially lead to problems in behavior, health, and fertility (Cyr and Romero 2009; Sheriff *et al.* 2011; Wingfield and Romero 2001). Various methods exist for improving housing conditions of zoo animals to minimize potential stress. Environmental enrichment, such as adding soil and vegetation or providing animals with special feeding devices, toys, and other novel objects, have become common practice in most zoos (Swaisgood and Sheperdson 2005; Vasconcellos *et al.* 2009) and have been shown to reduce stress hormone (glucocorticoid) output (Boinski *et al.* 1999; Buijs *et al.* 2011; Moreira *et al.* 2007; Pirovino *et al.* 2011). Moreover, many modern keeping facilities provide hiding places that allow animals to withdraw from the sight of conspecifics, visitors, or keepers.

Large crowds of visitors are an important source for stress in captive primates (reviewed in Davey 2007). For example, in zoo-housed western lowland gorillas (*Gorilla gorilla gorilla*) high visitor density correlated positively with intragroup aggression, stereotypies, and autogrooming (Wells 2005) as well as with self-scratching (Carder and Semple 2008). Another study has shown that Bornean orangutans (*Pongo pygmaeus*) at Chester Zoo, England, were disrupted by groups of visitors (Birke 2002). In addition, other factors have been reported to affect fecal glucocorticoid metabolite (fGCM) concentrations in primates such as sex (Honess and Marin 2006), age (Seraphin *et al.* 2008; Van Cauter *et al.* 1996), and changes in group composition (Clarke *et al.* 1995; Heistermann *et al.* 2004).

The welfare of captive animals could be further improved if zoos were to place emphasis on replicating the social conditions a species encounters in the wild when planning zoo facilities. For example, group size and group composition resembling the natural social condition of a species can have positive effects on stress hormone levels (Clarke *et al.* 1995; Reimers *et al.* 2007). In this respect, improvements are especially needed in the housing systems of orangutans. Both orangutan species, the Sumatran orangutan (*Pongo abelii*) and the Bornean orangutan, are usually kept under conditions similar to those for gorillas (*Gorilla* spp.): Several adult females and immatures share an enclosure with one adult male. Although such housing fits the natural social structure of gorillas, it is unnatural for orangutans, which live semisolitarily in the tropical rain forests of Borneo and Sumatra in an individual-based fission–fusion social system (van Schaik 1999). Adult females generally move through their home ranges only with their dependent offspring, and adult males roam their ranges mainly alone. Larger groups may form for feeding but are only temporary, and adult females and adult males maintain prolonged association only during consortships (van Schaik *et al.* 2009). However, the possibilities for an individual to avoid proximity to conspecifics

are usually extremely limited under captive housing conditions. These conditions may potentially lead to social stress, particularly for Bornean orangutans, which are adapted to an even more solitary lifestyle than their Sumatran sister species (Delgado and van Schaik 2000; van Noordwijk *et al.* 2009). In the wild, Bornean orangutans form close associations less frequently and generally live at lower densities than Sumatran orangutans (van Schaik *et al.* 2009). A zoo study documented that captive Bornean orangutans respond to living in larger groups with higher glucocorticoid concentrations, unlike their Sumatran counterparts, suggesting more pronounced susceptibility to social stress (Weingrill *et al.* 2011). This held true for both males and females. When the orangutans were housed alone, individuals of both species showed similar stress hormone values (Weingrill *et al.* 2011). The group size effect found in orangutans is in contrast to the findings of studies with gregarious primates. When isolated, captive gorilla males and New World monkeys had elevated glucocorticoid concentrations compared to baseline concentrations measured in their social group (Hennessy 1997; Mendoza *et al.* 2000; Stoinski *et al.* 2002).

Apenheul Primate Park in Apeldoorn, Netherlands, uses a housing approach that takes the natural fission–fusion social system of orangutans into account, housing 14 Bornean orangutans in 4 different subenclosures in frequently varying combinations. We investigated the extent to which this housing approach affects stress levels in the orangutans by assessing hormonal and behavioral indicators of stress. The measurement of fGCM is widely used as a non-invasive tool to investigate adrenocortical activity and thus physiological stress load (Heistermann *et al.* 2006; Lane 2006; Millspaugh and Washburn 2004; Rangel-Negrin *et al.* 2009). Behavioral approaches also serve as a noninvasive way to assess stress in primates (Honess and Marin 2006). Self-directed behavior such as autogrooming or scratching has been interpreted as displacement behavior due to frustration or anxiety (Aureli *et al.* 1989; Carder and Semple 2008; Honess and Marin 2006; Maestripieri *et al.* 1992; Troisi 2002) and used as a measure of stress, e.g., in olive baboons (*Papio hamadryas anubis*: Higham *et al.* 2009), in chimpanzees (*Pan troglodytes*: Fraser *et al.* 2008), and in orangutans (Elder and Menzel 2001). The few studies that have explored the relationship between glucocorticoid concentrations and rates of self-directed behavior in primates have generated ambiguous results: Some have found a positive correlation between the two measures (Peel *et al.* 2005), whereas others have not (Ellis *et al.* 2011; Higham *et al.* 2009; Ulyan *et al.* 2006).

Our first aim was to compare physiological indicators of stress, i.e., glucocorticoid output, in orangutans housed in a fission–fusion housing system with those in conventional housing systems. We predicted that orangutans living in fission–fusion conditions would show lower concentrations of stress hormones than those living in conventionally managed groups, especially if group size is large. Our second aim was to evaluate whether the presence of visitors had an effect on physiological and behavioral stress in the orangutans living in Apenheul's fission–fusion housing system. We predicted that levels of physiological and behavioral indicators of stress would rise on days with high visitor numbers. Our third aim was to examine the relationship between fGCM concentrations and self-directed behavior. Under the assumption that both measures quantify stress levels of orangutans we predicted that mean proportions of self-directed behavior and mean values of fGCM concentrations would correlate positively over the entire study period. To our knowledge, no other study has

investigated the interaction between these two measures in orangutans. Finally, we assessed whether other factors, such as sex, age, and change in group composition, affected fGCM concentrations or behavior.

Methods

Study Subjects and Housing

We conducted this study in Apenheul Primate Park Apeldoorn, Netherlands, from February 14, 2011 to August 1, 2011. The zoo was open to the public from March 30 until after the end of the study. During the study period 14 Bornean orangutans lived at Apenheul: one fully developed adult male with cheek flanges, two young adult males (not fully developed), seven females, and four immatures (one male, three females; Table I). We classified the individuals according to their age at the beginning of the behavioral observation period (May 9, 2011) and defined an adult as an orangutan older than 9 yr, as males and females living in zoos reproduce at this age (Weingrill *et al.* 2011).

The subjects were housed in four different subenclosures and the group compositions of the individuals living in the different subenclosures varied regularly. In the morning the orangutans were fed alone or in groups of two in eight separation rooms while the zookeepers cleaned the inside enclosures. Afterward the keepers released each orangutan into one of four possible subenclosures (A1, B1, C1, or D1; Fig. 1). The keepers tried to determine the orangutans' preferences by letting them choose the

Table I Sex, year of birth, age, birth type and location, rearing type, total follow time, and the number of fecal samples collected from Bornean orangutans at Apenheul Primate Park in Apeldoorn, Netherlands, February 2011–August 2011

Animal	Sex	Year of birth	Age	Birth type	Birth location	Rearing	Follow time (h)	No. of fecal samples
Karl	Male	1960	51	Wild born	Borneo	Parent	18.70	25
Radja	Female	1962	49	Wild born	Borneo	Parent	18.23	11
Silvia	Female	1965	46	Wild born	Borneo	Parent	17.50	13
Sandy	Female	1982	29	Captive born	Jersey	Unknown	19.03	13
Josje	Female	1992	18	Captive born	Rotterdam	Parent	16.93	13
Wattana	Female	1995	15	Captive born	Antwerp	Hand	16.93	28
Katja	Female	1997	13	Captive born	Rotterdam	Parent	18.17	15
Binti	Female	2000	10	Captive born	Antwerp	Parent	19.13	1
Amos	Male	2000	10	Captive born	Usti	Parent	18.27	25
Willie	Male	2002	9	Captive born	Apeldoorn	Parent	18.27	2
Samboja	Female	2005	6	Captive born	Apeldoorn	Parent	—	—
Dayang	Female	2005	6	Captive born	Apeldoorn	Parent	—	1
Merah	Female	2006	5	Captive born	Apeldoorn	Parent	—	2
Kawan	Male	2010	1	Captive born	Apeldoorn	Parent	—	—

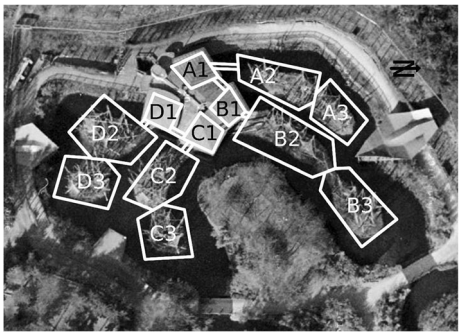

Fig. 1 Location of the four inside enclosures A1, B1, C1, and D1 and the connected islands A2, A3, B2, B3, C2, C3, D2, and D3 for Bornean orangutans at Apenheul Primate Park in Apeldoorn, Netherlands, February 2011–August 2011.

subenclosure actively or by taking their body language into account, e.g., an individual sitting in front of a subenclosure, and released them into the corresponding compartment by opening sliding doors. The subgroups then remained the same until the following morning. During the study period the number of adult individuals kept as a subgroup ranged from one to four, with the keepers varying the combination every few days. An island complex (with two islands) was connected to each inside subenclosure. Occasionally, large subgroups had access to two inside subenclosures and two island complexes.

The four inside enclosures had the same dimensions (surface area: *ca.* 58 m^2). Along the walls of the inside enclosures, a 1.5 m wide gallery was located 4 m above the ground. The ground level of the inside visitor room was located at the same height as the gallery, thus 4 m above the ground of the inside enclosures. Each inside enclosure had two sliding doors to the eight small separation rooms in the inner part of the building where the orangutans fed in the morning. Hay or wood shavings were always available as nesting material for the orangutans. Each inside enclosure was equipped with an *ad libitum* water source. The enclosures were provided with a climate control unit, which kept air humidity at 60–70 %. The island complexes served as the outdoor enclosures of the orangutans (mean surface area of one island: *ca.* 300 m^2). Each island was covered with grass and equipped with 10–20 tree trunks anchored in the ground.

Collection of Behavioral Data

We carried out behavioral observations between 09:30 h and 17:00 h in May and June and between 09:30 h and 18:00 h in July and August (for logistical reasons behavioral observation was possible only from May 8). Subjects had access to the outdoor enclosures throughout this period. M. Amrein collected all behavioral data. Behavioral data collection focused on all subadult and adult individuals, i.e., those older than 8 yr ($N = 10$; Table I). We used focal animal instantaneous sampling (Altmann 1974): We observed the focal individual for 40 min from the visitor area and recorded the activity of the subject every 2 min. We used a supplemented version of the standard sampling protocol published by the Anthropological Institute and Museum, University of Zurich, Switzerland (http://www.aim.uzh.ch/Research/orangutannetwork.html) to assign the observed activity to a category. Our main focus was on self-directed behavior, which

we used as a behavioral measure of stress and which included scratching (rhythmic flexion of fingers or toes, or up and down movement of wrists or ankles, so that the fingers or toes are rubbed gently across the individual's own fur or skin) and autogrooming (picking with fingers or mouth through the individual's own fur or skin while looking at this). The recorded activity was the one the orangutan showed during the first 5 s of each 2-min interval. If several consecutive activities occurred during those 5 s we noted only the behavior observed for the longest time during the 5 s.

We followed focal individuals in a random order but switched focal compartment only when every individual of the same subenclosure had been followed for one complete 40-min focal animal sampling period. After the end of each behavioral sampling period, we recorded the number of visitors around the orangutan enclosures. We recorded data directly into Microsoft Excel, using an HP netbook (HP Mini 210-2230ez).

For each observation day we calculated the mean proportion of visitors around the orangutan enclosures and compared it with the total number of visitors recorded officially by Apenheul Primate Park. The two measures correlated strongly and highly significantly (Spearman rank correlation: $N = 15$, $r = 0.854$, $P < 0.001$). For further analyses we thus used the official number of visitors because more data were available for this measure.

Collection of Hormonal Data

We measured glucocorticoid metabolites from fecal samples using the methods described in Weingrill *et al.* (2011). In most cases, the keepers collected samples from the separation rooms where the subjects were fed individually in the morning (collection was possible after the orangutans had left the rooms). However, the keepers collected 38 % of samples later during the day when they observed defecation and the orangutans changed compartments shortly after allowing keepers to collect the sample. We collected roughly 2–3 g of freshly defecated material into a plastic tube, labeled it with the subject ID, date, and time of defecation and stored it immediately in a freezer at *ca.* –20°C. We did not collect samples contaminated with urine. We collected the vast majority of samples (>90 %) within 20–30 min after defecation; no sample was collected >2 h after defecation. A study in gorillas using the same glucocorticoid assay as applied in the present study found no significant degradation of fGCM concentrations within the first 4 h post-defecation (Shutt *et al.* 2012).

In total, we collected 149 samples: 52 from adult males, 94 from adult females, and 3 from immature females. In three cases we collected two samples from the same individual on the same day. For the analysis we used mean values for each individual for each day. No samples were available for two of five immature orangutans. We recorded time of defecation for 121 samples from 12 individuals. Of these, we collected 75 in the morning and 46 in the afternoon (range 09:00 h–17:30 h, median sample time 09:45 h). To test for potential time of day effects in fGCM concentrations we fitted a linear mixed effects model (LMM) for all samples for which time was known. We entered fGCM concentration as a dependent variable, subject identity as random effect, and time of defecation as fixed effect. According to this model time of day was not a significant predictor for fGCM excretion ($N = 121$, $\beta_{MCMC} = -561.7$, $P_{MCMC} = 0.146$),

a finding in accordance with Weingrill *et al.* (2011). The earlier study also found no cycle-stage effect on fGCM concentrations in female orangutans (Weingrill *et al.* 2011), so we treated samples from cycling females in the same way as samples from nonlactating females for which we did not know cycle stage. We had too few samples of cycling females per month to allow us to determine cycle-stage effects on fGCM and behavior in females. One female was pregnant. We entered fGCM concentration as a dependent variable, subject identity as random effect, and pregnancy status as fixed effect in a LMM with 94 samples from 9 females housed in the fission–fusion system. Pregnancy was not a significant predictor for fGCM excretion (N = 94, β_{MCMC} = 524.5, P_{MCMC} = 0.115), so we pooled samples from the pregnant female with samples from nonpregnant females.

Hormone Assays and Interpretation

We transported all frozen fecal samples in a cooled Styrofoam box to the hormone laboratory of the German Primate Center (DPZ) in Göttingen, Germany. We freeze-dried, pulverized, and extracted an aliquot (0.05–0.07 g) of the fecal powder with 80% methanol (3 ml) as specified in detail by Heistermann *et al.* (1995). We measured glucocorticoid concentrations in fecal samples using a group-specific enzyme immunoassay (EIA) for 11ß-hydroxyetiocholanolone designed to measure 3α,11β-dihydroxylated cortisol metabolites (Ganswindt *et al.* 2003; Heistermann *et al.* 2006). Previous studies have used this assay successfully to monitor adrenocortical activity in several primate species of all major taxa, including great apes (Fichtel *et al.* 2007; Girard-Buttoz *et al.* 2009; Heistermann *et al.* 2006; Ostner *et al.* 2008; Pirovino *et al.* 2011). The assay has also been validated for use in orangutans (Weingrill *et al.* 2011). We diluted fecal extracts 1:60 with assay buffer before analysis and measured samples in duplicates in the EIA according to the method described in detail by Heistermann *et al.* (2004). We re-measured samples when optical density values between duplicates exceeded a coefficient of variation of 7%. Intra- and interassay coefficients of variation of high- and low-concentrated quality controls were 5.7 % (high) and 7.2 % (low) and 9.7 % (high) and 14.9 % (low), respectively.

The excretion lag of cortisol metabolites measured in fecal samples is *ca.* 48 h for orangutans (Weingrill *et al.* 2011). Accordingly, we applied an excretion lag of 2 d, i.e., we assigned the fGCM value deriving from a fecal sample to the date 2 d before the day of collection. This is consistent with other studies of primate endocrinology (Heistermann *et al.* 1996; Higham *et al.* 2009). We collected most of the fecal samples in the morning and usually carried out behavioral observations between 09:30 h and 18:00 h. For example, if we collected a fecal sample at 10:00 h, the hormone concentrations in this sample corresponded with activity levels an individual showed *ca*. 40–48.5 h earlier.

For comparison of our hormonal data from individuals living in a fission–fusion housing system with data from individuals living in permanent groups, we used a dataset with fGCM concentrations determined in 46 Bornean orangutans housed in 16 different European zoos (Weingrill *et al.* 2011). We had no behavioral data for these other individuals. In the fission–fusion subjects, adult group size (defined as the number of individuals housed in the same subenclosure that were older than 9 yr) ranged from one to four; in the other zoos adult group size ranged from one to five.

Data Treatment and Analysis

As we found no significant influence of time of day on fGCM concentrations, we included all samples in further analyses. We used LMMs to investigate which factors affected fGCM excretion. These allowed both random effects and fixed effects to be entered in the model and also to control for repeated measures by including animal identity as a random variable in the model. We entered fGCM concentrations as a dependent variable. To investigate which factors affected fGCM excretion in orangutans housed in different European zoos (including Apenheul) we applied a model with housing system (permanent groups or fission–fusion system), sex, adult group size, and age as fixed effects, in which individuals in a zoo were treated as a nested random effect. For this purpose we used 434 samples from 58 individuals housed in 17 different European zoos (including Apenheul). To investigate which factors affected fGCM excretion in orangutans living in the fission–fusion housing system we used a model with subject identity as a random effect and adult group size (number of adult individuals in a subgroup), sex, visitor number, time after a change in group composition (early: 1–5 d after a change in group composition, late: >5 d after a change in group composition), and age as fixed effects. For this purpose we used 146 samples from 12 individuals (10 adults and 2 immatures) housed in the fission–fusion system.

We used LMMs with subject identity as a random effect to investigate factors which affected stress-related behavior of the orangutans housed in the fission–fusion housing system. We entered scratching and autogrooming as dependent variables, and adult group size, sex, visitor number, time after change in group composition (as previously), and age of the individual as fixed effects. We calculated daily behavioral proportions from focal animal instantaneous sampling data of 10 individuals (denominator: total of activities that were counted for a given individual at a given day; numerator: specific activity, e.g., scratching or autogrooming). This yielded a dataset of 229 "animal days" for the analysis of factors related to behavioral stress.

We used LMMs with subject identity as a random effect, proportions of scratching and autogrooming as a fixed effect, and daily fGCM concentrations as dependent variable to test differences *within individuals*, i.e., to test whether an orangutan exhibiting high fGCM concentrations on a given day also exhibited high levels of self-directed behavior on this day, correcting for the time lag in fGCM excretion. We tested the relationship between the two measures *between individuals* using correlation analyses with mean values of fGCM concentrations (over the whole study period) and mean proportions of self-directed behavior (over the whole study period), i.e., we tested whether an individual exhibiting high mean values of fGCM concentrations over a longer period also exhibited high mean proportions of self-directed behavior over the same period.

We checked the assumptions of the LMMs (R function: lmer) using a graphic residual analysis. We used quantile–quantile plots to verify normality, Tukey–Anscombe plots to test for homoscedasticity, and leverage plots to find leverage points. If the assumptions did not hold we transformed the dependent variable. We did not exclude any outliers. For the analysis of fGCM data from Apenheul individuals no transformation was necessary. A $x^{0.75}$ transformation of the hormone values yielded the best residual distribution for the analysis of fGCM data from animals of all other European zoos. When the dependent variable consisted of a proportion we used an

arcsine transformation. Starting with full models with all interactions we generated final models using stepwise backward elimination of nonsignificant interaction terms ($P > 0.05$). We kept all main factors and significant interactions in the model. When there were significant interaction effects we did not interpret the corresponding main effects because main effect tests are not interpretable in the presence of interaction effects (Underwood 1997). The statistical significance of fixed effects is based on the highest posterior density (HPD) intervals obtained from Monte Carlo Markov Chain (MCMC) samplings with a chain length of 1,000,000 (Baayen 2008). We used Lilliefors (Kolmogorov–Smirnov) normality test and quantile–quantile plots to test for normality before performing correlation analyses to test the relationship between official visitor number and calculated visitor number (already reported in preceding text) and the relationship between fGCM concentrations and proportions of self-directed behavior. When the variables were normally distributed, we performed Pearson correlation analyses, whereas when they were not normally distributed, we used Spearman rank correlation analyses. We conducted all analyses using the arm, languageR, and nortest packages for R version 2.13.1.

Ethical Note

All data collection was noninvasive. Research procedures and animal husbandry adhered to the EAZA Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria and the WAZA Ethical Guidelines for the Conduct of Research on Animals by Zoos and Aquariums.

Results

Comparison of Fission–Fusion with Conventional Housing

Generally, fGCM concentrations in the subjects living in the fission–fusion housing system were in the same range as those seen in the other zoos, i.e., individuals housed in the fission–fusion system did not show substantially lower fGCM values. However, the effect of the interaction between housing system and adult group size approached significance ($P_{MCMC} = 0.051$, Table II, Fig. 2). Inspection of the highest posterior density interval (HPD95% interval: –61.2940 to 0.6623) moreover indicates that the interaction effect size was indeed significantly different from 0. This strongly suggests that the rate of increase of fGCM concentrations with increasing adult group size was less pronounced in the fission–fusion housing system than in the conventional housing system with permanent groups. Because the interaction between housing system and adult group size approached significance so closely we did not interpret these factors as main effects.

Factors Affecting fGCM Concentrations in Fission–Fusion Housing

The number of visitors had a highly significant positive effect on fGCM concentrations (Table III, Fig. 3). Adult group size, sex, time after a change in group composition, and age were not significantly associated with fGCM concentrations (Table III).

Table II Parameter estimates (β_{MCMC}) and associated significance values (P_{MCMC}) for all variables in the LMM of fGCM values for Bornean orangutans at Apenheul Primate Park in Apeldoorn, Netherlands, February 2011–August 2011 and 16 different European zoos ($N = 434$)

	β_{MCMC}	HPD95%$_{lower}$	HPD95%$_{upper}$	*t*-value	P_{MCMC}
(Intercept)	80.022	20.897	165.400	1.745	0.012
Housing	73.578	–56.272	185.218	1.026	0.251
Sex	–5.427	–36.696	22.690	–0.268	0.652
Group size	37.137	10.738	54.855	2.715	0.006
Age	0.605	–0.720	1.594	0.786	0.475
Housing: Group size	–33.562	–61.294	–0.662	–1.969	0.051

MCMC = Monte Carlo Markov Chain; HPD = highest posterior density interval.

Factors Influencing Self-Directed Behavior in Fission–Fusion Housing

We found a significant interaction between number of visitors and age on proportions of scratching (Table IV, Fig. 4). Specifically, the rate of increase of scratching proportions with growing numbers of visitors was higher in younger individuals than in older ones. Adult group size, sex, and time after a change in group composition were not significantly associated with proportions of scratching (Table IV). Because there was an interaction effect between number of visitors and age we did not interpret these factors as main effects.

There was also a significant interaction between number of visitors and age on proportions of autogrooming (Table V, Fig. 5). However, the effect was in the opposite direction to that for scratching, i.e., the rate of increase of autogrooming proportions with growing numbers of visitors was higher in older individuals than in younger ones.

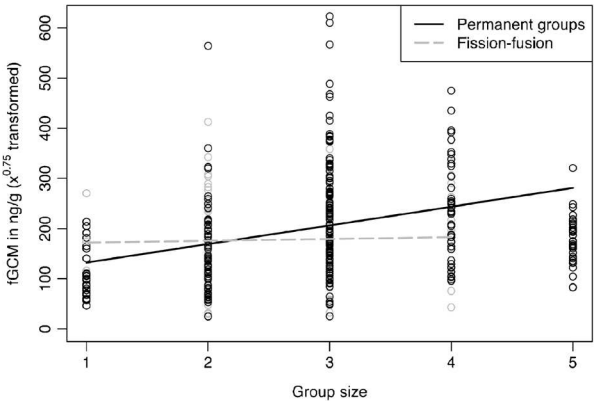

Fig. 2 Relationship between fGCM concentrations and adult group size in fission–fusion housing system (gray, dashed line, range 1–4) and in conventional housing systems with permanent groups (black, solid line, range 1–5). The lines represent the LMM predictions for the interaction between the two variables. Data for fission–fusion housing come from Bornean orangutans at Apenheul Primate Park in Apeldoorn, Netherlands, February 2011–August 2011; data for conventional housing system come from Bornean orangutans housed in 16 different European zoos (Weingrill *et al.* 2011).

Table III Parameter estimates (β_{MCMC}) and associated significance values (P_{MCMC}) for all variables in the LMM of fGCM values for Bornean orangutans at Apenheul Primate Park in Apeldoorn, Netherlands, February 2011–August 2011 ($N = 146$)

	β_{MCMC}	$HPD95\%_{lower}$	$HPD95\%_{upper}$	*t*-value	P_{MCMC}
(Intercept)	994.637	505.074	1629.631	3.237	0.002
Group size	48.122	–96.865	206.131	0.625	0.498
Sex	–144.380	–612.484	248.385	–0.561	0.398
Visitors	0.106	0.041	0.168	3.343	0.001
Time after change	–132.276	–320.466	81.722	–1.322	0.190
Age	–5.343	–18.201	5.534	–0.780	0.244

MCMC = Monte Carlo Markov Chain; HPD = highest posterior density interval.

Adult group size, sex, and time after a change in group composition were not significantly associated with proportions of autogrooming (Table V). As for scratching, there was an interaction effect between number of visitors and age, so we did not interpret these factors as main effects.

Relationship Between fGCMs and Self-Directed Behavior

Neither LMM testing the relationship between fGCM and self-directed behavior *within animals* showed a significant relationship (scratching: $N = 146$, $\beta_{MCMC} = 0.000006$, $P_{MCMC} = 0.703$; autogrooming: $N = 146$, $\beta_{MCMC} = 0.000088$, $P_{MCMC} = 0.258$). However, correlation analyses of the relationship between fGCM and self-directed behavior *between individuals* revealed a significant correlation between scratching and fGCM concentrations but not between autogrooming and fGCM concentrations (scratching, Pearson correlation: $N = 10$, $r = 0.644$, $P = 0.044$; autogrooming, Pearson correlation: $N = 10$, $r = 0.493$, $P = 0.147$).

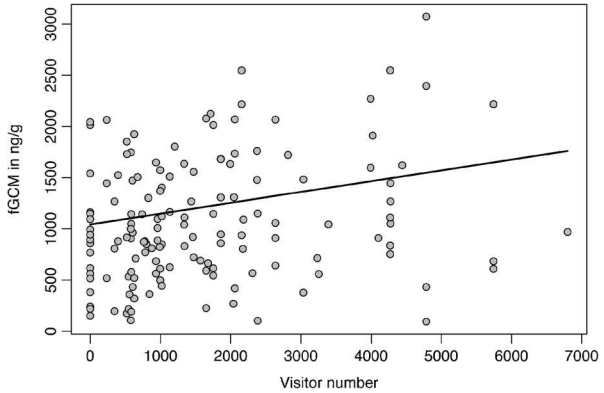

Fig. 3 Relationship between fGCM concentrations and visitor number revealed in the LMM of fGCM values for Bornean orangutans at Apenheul Primate Park in Apeldoorn, Netherlands, February 2011–August 2011.

Table IV Parameter estimates (β_{MCMC}) and associated significance values (P_{MCMC}) for all variables in the LMM of scratching proportions for Bornean orangutans at Apenheul Primate Park in Apeldoorn, Netherlands, May 2011–August 2011 ($N = 229$)

	β_{MCMC}	$HPD95\%_{lower}$	$HPD95\%_{upper}$	*t*-value	P_{MCMC}
(Intercept)	0.025875	–0.140170	0.194323	0.313	0.777
Group size	0.001120	–0.035114	0.039489	0.060	0.914
Sex	–0.026253	–0.134948	0.081750	–0.517	0.579
Visitors	0.000044	–0.000002	0.000092	1.832	0.067
Time after change	0.026601	–0.034671	0.082376	0.887	0.353
Age	0.002071	–0.001462	0.005984	1.133	0.250
Visitors: age	–0.000002	–0.000003	0.000000	–2.089	0.034

MCMC = Monte Carlo Markov Chain; HPD = highest posterior density interval.

Discussion

When kept in large groups orangutans housed in a fission–fusion system showed less of an increase in fGCM concentrations than individuals housed in permanent groups at other zoos. Although we only found a strong statistical trend for this interaction of group size and housing system, this suggests that the fission–fusion system reduced the group size effect on fGCM concentrations found in conventionally kept Bornean orangutans. Further, fGCM concentrations increased significantly with visitor numbers in the fission–fusion system and visitor number affected proportions of scratching and autogrooming. This effect was age-dependent, and mean proportions of scratching correlated positively with mean values of fGCM concentrations.

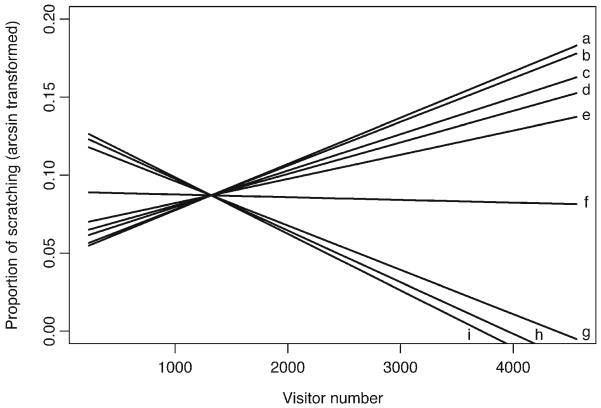

Fig. 4 Relationship between proportions of scratching and visitor number for all ages of the 10 analyzed individuals ($a = 9$ yr, $b = 10$ yr, $c = 13$ yr, $d = 15$ yr, $e = 18$ yr, $f = 29$ yr, $g = 46$ yr, $h = 49$ yr, and $i = 51$ yr). The lines represent the LMM predictions for the interaction between the two variables. Data come from Bornean orangutans and visitors at Apenheul Primate Park in Apeldoorn, Netherlands, May 2011–August 2011.

Table V Parameter estimates (β_{MCMC}) and associated significance values (P_{MCMC}) for all variables in the LMM of autogrooming proportions for Bornean orangutans at Apenheul Primate Park in Apeldoorn, Netherlands, May 2011–August 2011 ($N = 229$)

	β_{MCMC}	HPD95%$_{lower}$	HPD95%$_{upper}$	*t*-value	P_{MCMC}
(Intercept)	0.174025	–0.001266	0.351669	1.988	0.056
Group size	–0.015575	–0.053415	0.020047	–0.825	0.426
Sex	0.035498	–0.085888	0.161865	0.593	0.533
Visitors	–0.000027	–0.000074	0.000020	–1.154	0.258
Time after change	0.006862	–0.053292	0.067892	0.229	0.778
Age	–0.002668	–0.006783	0.001488	–1.316	0.203
Visitors: age	0.000002	0.000000	0.000003	2.536	0.013

MCMC = Monte Carlo Markov Chain; HPD = highest posterior density interval.

Comparison of Fission–Fusion with Conventional Housing

Our model revealed a strong trend indicating that the rate of increase of fGCM concentrations with increasing adult group size is smaller in Bornean orangutans kept in the fission–fusion housing system than in Bornean orangutans kept in a conventional housing system. The values for the individuals kept in the fission–fusion system are in line with the smaller subgroup sizes and far below the values that would be expected for the total group size of 10 adults. This result, based on physiological stress of orangutans in a cross section of European zoos, is in line with the outcome of other LMMs investigating the effect of adult group size on fGCM concentrations and proportions of self-directed behavior in orangutans housed in Apenheul's fission–fusion system: All three models (for fGCM concentrations, proportions of scratching, and proportions of autogrooming) showed that group size did not significantly affect the physiological (fGCM) and behavioral measures (self-directed behavior) of stress in

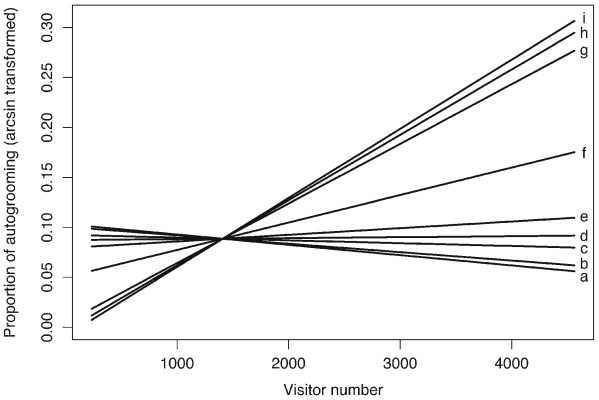

Fig. 5 Relationship between proportions of autogrooming and visitor number for all ages of the 10 analyzed individuals ($a = 9$ yr, $b = 10$ yr, $c = 13$ yr, $d = 15$ yr, $e = 18$ yr, $f = 29$ yr, $g = 46$ yr, $h = 49$ yr, and $i = 51$ yr). The lines represent the LMM predictions for the interaction between the two variables. Data come from Bornean orangutans and visitors at Apenheul Primate Park in Apeldoorn, Netherlands, May 2011–August 2011.

animals living in the fission–fusion housing system. Even though the animals are likely to be aware of their nearby conspecifics and can hear or see them on the other islands, they respond as though they are not affected by them. Thus, individuals living in a fission–fusion system seem to be less affected by social stressors associated with larger group sizes than those living in permanent groups.

Permanently solitary orangutans and orangutans in small permanent groups have the lowest fGCM concentrations (Weingrill *et al.* 2011). It appears, however, that the stressful effect of living in a large permanent group (Weingrill *et al.* 2011) is absent for orangutans in the fission–fusion system we studied. This is unlikely to be due to differences between the temporary sizes of groups at Apenheul and the more permanent group sizes in other zoos, as the range of group sizes were comparable. It is more likely that the fission–fusion system allows keepers to change group compositions as soon as they notice social tension in groups and to reduce potential sources of social stress by modifying the group composition, which resembles the situation in the wild when individuals have the option to avoid social conflicts by moving away. fGCM concentrations and proportions of self-directed behavior were affected neither by the time since a change in group composition nor by the social interactions in the newly formed subgroups during the first 5 d after a change, indicating that this management procedure does not substantially increase the orangutans' social stress. Because we tested only one zoo with a fission–fusion housing system against 16 zoos with a conventional housing system, it is possible that other zoo-specific factors influenced this result. Therefore, our data should ideally be confirmed with more groups living in a fission-fusion social environment.

Effect of Visitors on Stress Indicators

While the size of the group and the changing of groups had no effect on stress hormone concentrations in Apenheul's orangutans, visitors had a highly significant effect on adrenocortical activity. These findings are in line with results from several other studies, e.g., with blackbucks (*Antelope cervicapra*: Rajagopal *et al.* 2011) and with spider monkeys (*Ateles geoffroyii rufiventris*: Davies *et al.* 2005). Urinary cortisol concentrations (but not saliva cortisol concentrations) likewise increased significantly with higher visitor number in captive orangutans at Chester Zoo (Wehnelt *et al.* 2004). Moreover, our data are in accordance with recent findings on habituated wild orangutans in an ecotourism project that also responded to the presence of visitor groups with an increase in the concentration of fecal stress hormones (Muehlenbein *et al.* 2012). The apparent stress-inducing effect of visitors on the focal individuals was also seen in our behavioral measure of stress, i.e., the level of self-directed behavior shown by the subjects. A similar result was found for wild Barbary macaques (*Macaca sylvanus*) in which self-scratching rates were positively related to the number of tourists at an ecotourism site in Morocco (Maréchal *et al.* 2011).

Our data indicate that young and old orangutans responded differently to increasing numbers of visitors. Scratching proportions increased more in younger individuals than in older ones, whereas autogrooming proportions increased more in older individuals than in younger ones. In captive red-capped mangabeys (*Cercocebus torquatus torquatus*), age correlates positively with the total rate of self-directed behavior, including yawning, self-scratching, self-grooming, and body

shaking (Reamer *et al.* 2010). However, to our knowledge there is no study reporting an age effect regarding the specific type of self-directed behavior in primates, i.e., documenting that older individuals show different self-directed behavior than young ones. It may be that hygiene-related habits such as scratching and autogrooming change with age and that age also affects the use of these behaviors to cope with a stressful situation. Before any firm conclusions can be made, however, more studies are needed to examine whether individual self-directed behaviors in primates are age-related and whether this may, in turn, be linked to age-related differences in stress coping mechanisms. Self-directed behavior has been implicated with social stress and, in particular, anxiety, in primates (Carder and Semple 2008; Honess and Marin 2006; Maestripieri *et al.* 1992; Maréchal et al. 2011; Troisi 2002). Our study provides strong evidence that the same factor that leads to increased physiological stress, i.e., visitor number, also affects self-directed behavior.

The presence of visitors seems to represent a potential stressor to orangutans. It is, therefore, important that management should strive to reduce interactions between visitors and orangutans, as this may lead to a state of chronic stress, which can have marked negative effects on an animal's health and reproduction (Cyr and Romero 2009; Sheriff *et al.* 2011; Wingfield and Romero 2001). Several studies have shown that visitor presence is a stressor for captive primates, from which these individuals try to retreat if possible (Pirovino *et al.* 2011; Smith and Kuhar 2010). Colored glass elements in the windows prevent the orangutans from seeing people standing in the visitor room to some extent in Apenheul. The orangutans also have jute sacks, hay, or wood wool to cover their body partially. However, the orangutans have limited possibilities to retreat from the sight of visitors and there are no areas where they are out of public view during the day. Moreover, the floor level of the public area is elevated in comparison with the ground level of the orangutan enclosures, allowing visitors to observe the orangutans at the same height. This might be problematic, as arboreal primates seem to be particularly sensitive to unrestricted visual contact with humans at eye level (Davis *et al.* 2005; Pirovino *et al.* 2011; Smith and Kuhar 2010). Orangutans may interpret gazing by visitors as threatening because it is uncommon for orangutans to look directly at another individual. Orangutans typically use sidelong glances, i.e., only the eyes are turned to an object of interest but the head is not (Kaplan and Rogers 1999, 2002). For example, an adult Bornean male at Chester Zoo (UK) responded with banging and calling to prolonged staring by human males (Birke 2002).

Being aware that visitors are a source of stress for animals, zoos are confronted with the conflict between providing their animals with the best environment for their welfare and serving their institution's main purpose: giving visitors the possibility to observe animals. This dilemma could be reduced by lowering the height of viewing areas or increasing the height of cages to reduce the stressful effect of visitors (Pirovino *et al.* 2011). This may not be feasible in Apenheul. However, providing the orangutan enclosures with additional hiding facilities, such as areas covered with camouflage nets, might reduce the individuals' stress response, as demonstrated by a study implementing sight barriers for captive western lowland gorillas (Blaney and Wells 2004).

Sex had no significant effect on fGCM concentrations or proportions of self-directed behavior in our focal individuals. Given that data come only from three males and

seven females, this result should be treated with caution. However, it is in line with previous findings, which also found that male and female fGCM concentrations are comparable within Bornean as well as Sumatran orangutans (Weingrill *et al.* 2011). We also found no age effect on fGCM concentrations. In contrast, glucocorticoid concentrations have been reported to be higher in older individuals in humans, chimpanzees, brown spider monkeys (*Ateles hybridus*), and red howlers (*Alouatta seniculus*) (Rimbach *et al.* 2013; Seraphin *et al.* 2008; Van Cauter *et al.* 1996). An earlier study on orangutans found a small significant increase in fGCM concentrations with increasing age in females but not in male orangutans (Weingrill *et al.* 2011). More studies are therefore needed to assess the extent to which age is a significant predictor of adrenocortical activity in orangutans.

Relationship Between fGCM and Self-Directed Behavior

fGCM concentrations did not show a significant relationship with the proportion of time spent scratching per day. In other words, subjects did not show both high fGCM concentrations and high scratching proportions at the same time in a stressful situation. However, over the entire study period, we found a significant positive correlation between mean proportions of scratching and mean values of fGCM concentrations. This indicates that certain individuals are generally more stressed than others. The same pattern has been reported for autogrooming in captive Barbary macaques (Edwards *et al.* 2013).

It is possible that self-directed behavior is a coping strategy to reduce physiological stress response (Higham *et al.* 2009; Maestripieri *et al.* 1992; Watson *et al.* 1999). If this is true, individuals with high concentrations of glucocorticoids should show even higher concentrations if they would not have been able to make use of the calming effect of self-directed behavior such as scratching. This may explain why rates of self-directed behavior and fGCM concentrations are often found not to correlate and why the few studies that explored this relationship have generated ambiguous results. Using day-by-day data, a positive correlation between self-directed behavior and fGCM concentrations has been found in captive western lowland gorillas (Peel *et al.* 2005). However, no relationship between the two measures could be detected in captive brown capuchins (*Cebus apella*: Ulyan *et al.* 2006) or wild olive baboons (*Papio hamadryas Anubis*: Ellis *et al.* 2011; Higham *et al.* 2009). Further studies quantifying self-directed behavior and fGCM concentrations are needed to resolve this open question.

Conclusion

This is one of the first studies to investigate hormonal and behavioral measures of stress simultaneously in zoo-housed Bornean orangutans. We showed that a group size effect on adrenocortical activity found in Bornean orangutans kept in a conventional housing system with permanent groups is missing from the fission–fusion housing system in Apenheul Primate Park. We also found no group size effect on self-directed behavior associated with behavioral stress. We showed that large numbers of visitors increase fGCM concentrations and affect proportions of self-directed behavior in Bornean orangutans housed at Apenheul. Further, we found that individual mean proportions

of scratching correlated positively with mean values of fGCM concentrations. Our findings support the hypothesis that a fission–fusion system can mitigate the potential stress of group living for Bornean orangutans in captivity. However, visitors were an important source for stress in Bornean orangutans. This should be considered in the planning or restructuring of captive housing facilities in the future, as it may help to improve welfare and even breeding success of this endangered great ape species. Moreover, our results support the general finding that it is worthwhile to improve housing conditions of captive primates as well as other zoo animals. Environmental enrichment in zoos should not only encourage increase in activity among the animals, but should also allow them to live in the species-specific social organization found under natural, wild conditions, including giving them the opportunity to retreat from conspecifics and human visitors.

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